# Interleukin-10-Treated Dendritic Cells Modulate Immune Responses of Naive and Sensitized T Cells *In Vivo*

Gabriele Müller,[1] Anke Müller,[1] Thomas Tüting, Kerstin Steinbrink, Joachim Saloga, Claudia Szalma, Jürgen Knop, and Alexander H. Enk

Department of Dermatology, University of Mainz, Mainz, Germany

**Interleukin-10 is a pleiotropic cytokine known to have inhibitory effects on the accessory functions of dendritic cells. *In vitro*, interleukin-10 converts immature dendritic cells into tolerizing antigen-presenting cells. To assess whether interleukin-10-treated dendritic cells exert tolerizing effects *in vivo*, $CD4^+$ T cells from DO11.10 ovalbumin–T cell receptor transgenic mice were transferred to syngeneic BALB/c recipients. Recipient animals were treated with ovalbumin-pulsed/unpulsed, interleukin-10-treated/untreated $CD11c^+$ dendritic cells thereafter and ovalbumin-specific proliferation of lymph node cells was assessed by restimulation with the peptide *in vitro*. In prophylactic experiments, recipients received naive $CD4^+$ DO11.10 T cells and were immunized with ovalbumin$^{323–339}$ peptide in incomplete Freund's adjuvant after treatment with various subtypes of dendritic cells. Strong ovalbumin-specific proliferation was observed in animals immunized with control ovalbumin-dendritic cells. Minimal proliferation was found in mice treated with ovalbumin-pulsed, interleukin-10-treated dendritic cells. In therapeutic experiments, preactivated $CD4^+$ DO11.10 T cells were transferred, and recipients were treated with dendritic cells as described. Ovalbumin-specific proliferation was strong in recipients treated with ovalbumin-dendritic cells. $CD4^+$ T cell proliferation from ovalbumin–interleukin-10–dendritic cell treated animals was below background. When delayed type hypersensitivity reactions in the footpads of prophylactically or therapeutically vaccinated animals were tested, mice treated with ovalbumin–interleukin-10–dendritic cells showed no footpad swelling compared with controls. Rechallenge with the antigen *in vitro* and *in vivo* did not alter the inhibitory effect of interleukin-10-treated dendritic cells. Thus, interleukin-10-treated dendritic cells inhibit ovalbumin-specific immune responses in naive and sensitized mice.** ***Key words: dendritic cells/interleukin-10/ovalbumin. J Invest Dermatol 119:836–841, 2002***

Dendritic cells (DC) are potent immunostimulatory cells whose capabilities to process and present antigen are unmatched in the immune system (Banchereau and Steinman, 1998). Besides serving as potent immunostimulatory cells, DC can be converted to tolerance-inducing cells under certain conditions (Schuler and Steinman, 1985; Steinman and Young, 1991; Young *et al*, 1992). One of the factors known to affect DC in such a way that they become tolerizing antigen-presenting cells (APC) is interleukin-10 (IL-10) (de Waal *et al*, 1992a). It was shown by us and others previously that IL-10 is able to affect the expression of major histocompatibility complex (MHC) and costimulatory molecules such as CD86 on DC (Steinbrink *et al*, 1997). Although the original work was performed in murine Langerhans cells (Enk *et al*, 1993; 1997; Dummer *et al*, 1995; 1996), similar results were obtained in various subtypes of murine and human DC. As an example, IL-10-treated human-blood-derived DC induced anergy in $CD4^+$ and $CD8^+$ clonal or naive T cells. Induction of anergy in melanoma-antigen-specific T cell lines also resulted in a failure of these cytotoxic T lymphocytes to lyze tumor cells (Pisa *et al*, 1992; Gastl *et al*, 1993; Merlo *et al*, 1993; Smith *et al*, 1994; Kim *et al*, 1995; Steinbrink *et al*, 1999). Additionally, data generated in patients with malignant melanoma showed that progressing metastases in contrast to regressing metastases contained high amounts of IL-10 (Enk *et al*, 1997). This IL-10 was derived from melanoma cells and inhibited the expression of CD86 on local DC. Therefore these DC were not only inhibited with regard to their potency to induce T cell proliferation, but also induced tolerance in the patients' T cells. In aggregate these data indicate that IL-10 serves as a factor that alters DC functions in such a way that these DC induce T cell tolerance. As tolerance induction might be a useful way to treat murine or human autoimmune diseases, we wondered whether IL-10-treated DC (DC-IL10) might also subserve tolerizing functions *in vivo*.

To address the question of downregulation of immune responses by DC *in vivo*, we used a system of ovalbumin (OVA)–T cell receptor (TCR) transgenic animals (DO11.10 mice) where 50%–70% of all naive peripheral $CD4^+$ T cells express an OVA-specific TCR (Murphy *et al*, 1990). This system provides the advantage that a large number of peptide-specific T cells can be readily stimulated with the appropriate antigen. To exclude an influence of newly developing transgenic T cells that were not affected by the DC

Manuscript received May 14, 2002; revised May 31,2002; accepted for publication June 9, 2002

[1]G. Müller and A. Müller contributed equally to this work.

Reprint requests to: Professor A. Enk, Department of Dermatology, Langenbeckstr. 1, 55131 Mainz, FRG; Email: enk@hautklinik.klinik.uni-mainz.de

Abbreviations: APC, antigen-presenting cell; BM-DC, bone-marrow-derived dendritic cell; DC, dendritic cell; OVA, ovalbumin; rmGM-CSF, recombinant mouse granulocyte–macrophage colony-stimulating factor; rmIL, recombinant mouse interleukin.

injected and to test transgenic T cells in a ''normal'' nontransgenic environment, $CD4^+$ T cells from transgenic animals were transferred *ex vivo* into syngeneic BALB/c mice (Kearney *et al*, 1994). We demonstrate that pretreatment of DC with IL-10 alters the APC functions of these cells in such a way that DC-IL10 are able to prevent and downmodulate activation of transgenic $CD4^+$ T cells *in vivo*. Therefore, DC-IL10 might provide a potent tool for the prevention and treatment of murine or human autoimmune diseases.

## MATERIALS AND METHODS

**Animals** BALB/c mice ($H\text{-}2^d$) and OVA-TCR DO11.10 mice (kindly provided by Dr. Dennis Loh, $H\text{-}2^d$, $OVA^{323–339}$ peptide) were bred in the animal facility of the Institute of Immunology in Mainz and used at 8–12 wk of age. For *in vivo* experiments five mice were used in each group (Murphy *et al*, 1990).

**Culture medium for DC** RPMI 1640 was supplemented with 100 IU per ml penicillin, 100 μg per ml streptomycin, $5 \times 10^{-5}$ M 2′-mercaptoethanol, 2 mM glutamine, 10 mM HEPES, and 1.5% heat-inactivated pooled BALB/c mouse serum. All cytokines used were recombinant mouse proteins. Final concentrations used in the cultures were 10 ng per ml for granulocyte–macrophage colony-stimulating factor (GM-CSF), 10 ng per ml for IL-4, and 30 ng per ml IL-10 (Schering-Plough).

**Antibodies and magnetic beads** The following antibodies were used anti-CD4 (clone GK1.5, ATCC, Rockville, MD), $rIgG_{2b}$, RB6-8C5 (clone Ly-6G/Gr-1, ATCC), $rIgG_{2a}$, class II $I\text{-}A^{b,d,q}$, $I\text{-}E^{d,k}$ (clone M5/114.15.2, ATCC), $rIgG_{2b}$, anti-CD80 (clone 16-10A1, PharMingen), anti-CD86 (clone GL1, PharMingen), anti-CD62L (clone MEL14, PharMingen).

For magnetic separation we used the following beads: CD4-microbeads (Miltenyi Biotec, Bergisch-Gladbach, Germany).

**Generation of fetal-bovine-serum-free bone-marrow-derived DC (BM-DC)** Murine BM-DC were grown according to published protocols in RPMI medium supplemented with 10 ng per ml recombinant murine GM-CSF (rmGM-CSF), 10 ng per ml recombinant murine IL-4 (rmIL-4), and 1.5% mouse serum in a final volume of 25 ml. At day 2 cells were treated with 30 μg per ml rmIL-10 or left untreated. On day 5 of culture, nonadherent cells were pulsed with 50 μg per ml OVA or left unpulsed. After 2 d cells were assessed by fluorescence-activated cell sorter (FACS) for expression of CD11c, MHC class II, and costimulatory molecules (CD80, CD86). Regular yields were > 70% $CD11c^+$ DC.

**Generation of epidermal cells** Mice were sacrificed and ears removed. After disinfection with 70% ethanol ears were split into dorsal and ventral halves using fine tweezers. Subcutaneous fat and cartilage were removed by scraping with the tweezers. Ear halves were digested in 0.5% trypsin/0.2 mM ethylenediamine tetraacetic acid for 30 min. The epidermis was removed easily and epidermal cells were washed out of the tissue. After incubation of the cells in RPMI medium supplemented with 100 IU per ml penicillin, 100 μg per ml streptomycin, $5 \times 10^{-5}$ M 2′-mercaptoethanol, 2 mM glutamine, 10% fetal bovine serum, and 30 μg per ml OVA overnight Langerhans cells were enriched by density centrifugation.

**Prevention of OVA-specific immune responses *in vivo* (Fig 1*A*)** $CD4^+$ T cells of naive DO11.10 mice were purified with magnetic beads (Miltenyi, 98% purity) and injected intravenously into naive BALB/c mice ($5 \times 10^6$ cells per mouse). Mice were injected intravenously with $5 \times 10^5$ DC treated with IL-10 or left untreated and pulsed with OVA or left unpulsed 48 h later. After a rest period of 8 d mice were sensitized by subcutaneous injection with 30 μg $OVA^{323–339}$ peptide in incomplete Freund's adjuvant (IFA). Ten days later mice were either sacrificed to obtain lymph node cells for *in vitro* analysis or injected into the footpad with 30 μg $OVA^{323–339}$ peptide in IFA for analysis of delayed-type hypersensitivity (DTH) reactions *in vivo*. The ratio of OVA-TCR transgenic $CD4^+$ T cells contained in lymph node cells was analyzed by FACS and equal amounts were used for restimulation. Proliferation of OVA-specific T cells restimulated with 5 μg per ml $OVA^{323–339}$ peptide was measured by $^3$H-thymidine incorporation. As tolerance control mice were injected once with 300 μg $OVA^{323–339}$ peptide intraperitoneally and lymph node cells were processed as described.

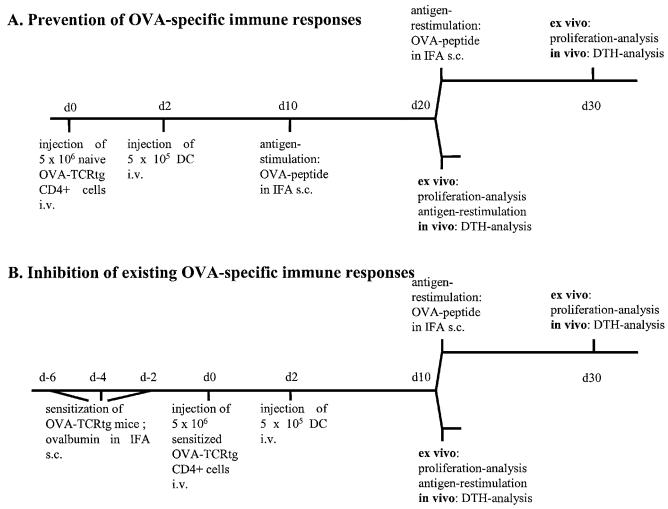


**Figure 1. Experimental systems.** (*A*) $CD4^+$ T cells of naive DO11.10 mice were purified and injected intravenously into naive BALB/c mice ($5 \times 10^6$ cells per mouse). Mice were injected intravenously with $5 \times 10^5$ DC 48 h later. After a rest period of 8 d mice were sensitized by subcutaneuos injection with 30 μg $OVA^{323–339}$ peptide in IFA. Ten days later mice were either sacrificed to obtain lymph node cells for *in vitro* analysis or injected into the footpad with 30 μg $OVA^{323–339}$ peptide in IFA for analysis of DTH reactions *in vivo*. (*B*) $CD4^+$ T cells of presensitized DO11.10 mice were injected intravenously into naive BALB/c mice ($5 \times 10^6$ cells per mouse). Mice were injected intravenously with $5 \times 10^5$ DC 48 h later. After a rest period of 8 d mice were either sacrificed to obtain lymph node cells for *in vitro* analysis or injected into the footpad with 30 μg $OVA^{323–339}$ peptide in IFA for analysis of DTH reactions *in vivo*. To analyze whether the effects seen were long lasting, some mice were rechallenged with antigen at day 10 (*A*) or day 20 (*B*) and DTH experiments and *in vitro* analysis were performed 10 d later.

**Inhibition of an existing OVA-specific immune response *in vivo* (Fig 1*B*)** DO11.10 mice were sensitized three times every other day with 5 mg OVA per mouse subcutaneously in IFA. $CD4^+$ T cells of these sensitized DO11.10 mice were isolated from lymph node cells (98% purity) and injected intravenously into naive BALB/c mice ($5 \times 10^6$ cells per mouse). Mice were injected intravenously with $5 \times 10^5$ DC treated with IL-10 or left untreated and pulsed with OVA or left unpulsed 48 h later. After a rest period of 8 d mice were either sacrificed to obtain lymph node cells for *in vitro* analysis or injected into the footpad with 30 μg $OVA^{323–339}$ peptide in IFA for analysis of DTH reactions *in vivo*. The ratio of OVA-TCR transgenic $CD4^+$ T cells contained in lymph node cells was analyzed by FACS and equal amounts were used for restimulation. Proliferation of OVA-specific T cells restimulated with 5 μg per ml $OVA^{323–339}$ peptide was measured by $^3$H-thymidine incorporation.

**Antigen restimulation *in vivo* and *in vitro*** For *ex vivo* restimulation analysis lymph node and spleen cells were prepared from all animals injected with DO11.10 cells and DC. $CD4^+$ T cells were isolated and stimulated with OVA-pulsed epidermal cells. Three days later the medium was supplemented with 2 U per ml rhIL-2. After 10 d $CD4^+$ cells were harvested and equal numbers of OVA-specific T cells were restimulated with OVA-pulsed epidermal cells. Proliferation was measured by $^3$H-thymidine incorporation.

For *in vivo* restimulation mice reconstituted with $CD4^+$ DO11.10 T cells and injected with DC as described were restimulated by subcutaneuos injection with 30 μg $OVA^{323–339}$ peptide in IFA. Ten days later mice were injected into the footpad with 30 μg $OVA^{323–339}$ peptide in IFA or IFA alone into the contralateral footpad for analysis of DTH reactions.

## RESULTS

**IL-10-treated DC inhibit immune responses of naive T cells *in vivo*** $CD4^+$ T cells from naive DO11.10 mice were injected into syngeneic BALB/c mice. Two days later, animals transplanted

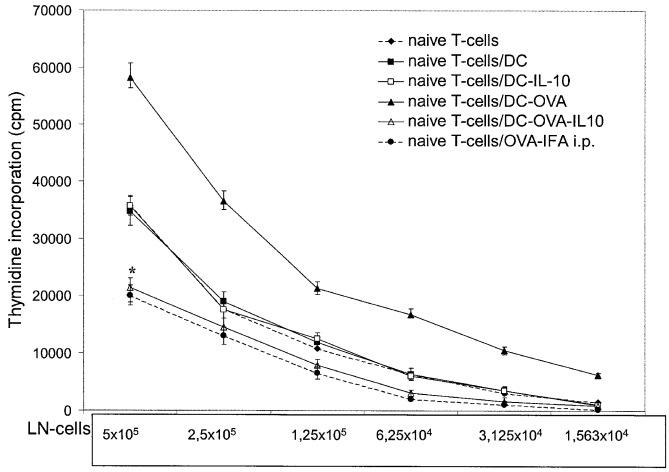


**Figure 2. IL-10 DC prevent induction of OVA-specific immune responses.** $CD4^+$ T cells from naive DO11.10 mice were transferred to syngeneic BALB/c mice. Two days later recipients were treated with either IL-10-treated or untreated, OVA-pulsed or unpulsed DC as described. Eight days later mice were immunized with $OVA^{323–339}$ in IFA subcutaneously and lymph node cells were prepared 10 d later. Proliferation was induced by addition of $OVA^{323–339}$ peptide to the cultures and thymidine incorporation was determined after 3 d. Results shown are representative of five experiments with five mice per group. Groups are mice receiving naive $CD4^+$ T cells and unpulsed DC (naive T cells/DC), mice receiving naive $CD4^+$ T cells and DC-OVA (naive T cells/DC-OVA), mice receiving naive $CD4^+$ T cells and DC-OVA-IL10 (naive T cells/DC-OVA-IL10), mice receiving naive $CD4^+$ T cells and DC-IL10 (naive T cells/DC-IL10) and mice receiving naive $CD4^+$ T cells and OVA peptide in IFA intraperitoneally (OVA-IFA i.p. = tolerance control). Error bars represent SEM. *$p < 0.05$ *versus* DC control. Statistical significance was determined by *t* test.

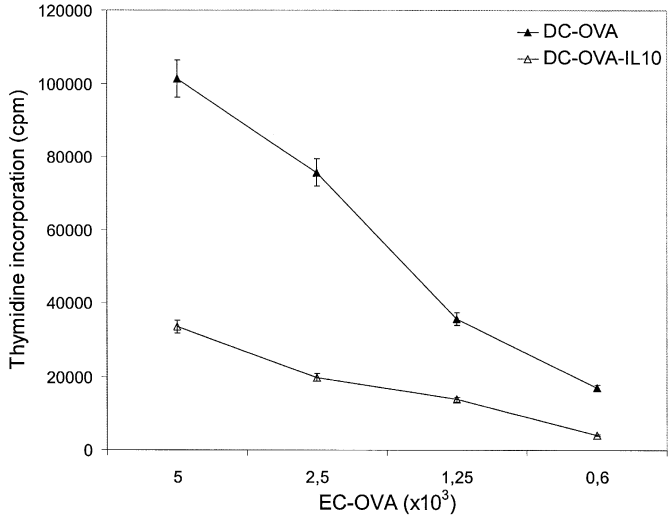


**Figure 3. OVA-pulsed IL-10-treated DC induce antigen-specific tolerance in naive T cells *in vivo*.** $CD4^+$ T cells from naive DO11.10 mice were transferred to syngeneic BALB/c mice. Two days later recipients were treated with either IL-10-treated or untreated, OVA-pulsed as described. Eight days later mice were immunized with $OVA^{323–339}$ in IFA subcutaneously and $CD4^+$ T cells were isolated from lymph node and spleen cells 10 d later. $CD4^+$ T cells were restimulated *in vitro* with OVA-pulsed epidermal cells. After a rest period and addition of IL-2 to cultures (2 U per ml) $CD4^+$ T cells were restimulated with OVA-pulsed epidermal cells and thymidine incorporation was determined after 3 d. Results shown are representative of five experiments with five mice per group. Groups are mice receiving naive $CD4^+$ T cells and DC-OVA and mice receiving naive $CD4^+$ T cells and DC-OVA-IL10. Error bars represent SEM.

with transgenic T cells were injected intravenously with $5 \times 10^5$ BM-DC from normal BALB/c mice that were either pulsed with OVA (DC-OVA) or pulsed with OVA and pretreated with IL-10 (DC-OVA-IL10). Control mice received DC that were pretreated with IL-10 (DC-IL10) or left untreated (DC). Prior to injection DC were analyzed by FACS staining. IL-10 treatment reduced the expression of both stimulatory (MHC II) and costimulatory molecules (CD80, CD86, CD40). Expression of the DC marker CD11c was not affected (data not shown). After a period of 8 d animals were stimulated with the immunogenic $OVA^{323–339}$ peptide by subcutaneous injection. Ten days later, total lymph node cells (containing APC) from these animals were prepared and restimulated with the peptide *in vitro*. The number of OVA-TCR transgenic T cells was determined by FACS and equal amounts were used for restimulation. Proliferation was measured after 3 d by thymidine incorporation. Whereas lymph node cells from mice treated with OVA-pulsed DC (DC-OVA) *in vivo* proliferated well to antigenic restimulation *in vitro*, there was only limited proliferation in the lymph node cells derived from animals treated with IL-10-treated OVA-pulsed DC (DC-OVA-IL10). This result is in concordance with the effects of DC-OVA-IL10 seen after stimulation *in vitro* (data not shown). The amount of proliferation detectable in this group was comparable to the amount of proliferation visible in the tolerance control group (lymph node cells from animals tolerized by intraperitoneal injection of immunogenic peptide in IFA) (**Fig 2**). Omission of antigen in the *in vivo* treatment resulted in proliferation rates similar to that observed with lymph node cells from naive animals. To analyze whether the inhibitory effects seen remained after antigen restimulation, $CD4^+$ T cells were isolated from mice injected with DC-OVA and DC-OVA-IL10, respectively, and were restimulated with OVA-pulsed epidermal cells. After 3 d the medium was supplemented with 2 U per ml IL-2. After a rest period equal numbers of OVA-TCR transgenic T cells were restimulated with OVA-pulsed epidermal cells. $CD4^+$ T cells from mice injected with DC-OVA-IL10 after reconstitution with naive $CD4^+$ DO11.10 T cells remained impaired in their capacity to proliferate (**Fig 3**). $CD4^+$ T cells isolated from mice injected with DC or DC-IL10 were driven into cell death by *ex vivo* restimulation.

These data indicate that IL-10-treated DC inhibit antigen-specific immune responses in naive $CD4^+$ transgenic T cells *in vivo*. This inhibition persists after multiple rounds of restimulation.

**IL-10-treated DC are able to suppress preexisting immune responses in transgenic T cells *in vivo*** DO11.10 mice were sensitized by subcutaneous injection of OVA in IFA. $CD4^+$ T cells from these animals were prepared as described and injected intravenously into naive syngeneic BALB/c recipients. Two days later recipients were treated with $5 \times 10^5$ BM-DC from BALB/c mice that had been pulsed with OVA or were left unpulsed, and that were either pretreated with IL-10 or left untreated. Eight days later lymph node cells from all groups of recipients were prepared and restimulated with the immunogenic peptide *in vitro*. The number of OVA-TCR transgenic $CD4^+$ T cells was determined by FACS and equal amounts were used for restimulation. Proliferation was measured by thymidine incorporation. As shown in **Fig 4**, lymph node cells from recipients treated with DC-OVA showed a strong proliferation upon stimulation with OVA peptide *in vitro* whereas lymph node cells from recipients treated with DC-OVA-IL-10 displayed 70% lower proliferation. In fact, proliferation of lymph node cells derived from DC-OVA-IL-10 injected mice was even lower than the proliferation of naive $CD4^+$ transgenic T cells (from untreated mice) that first encountered antigen presentation *in vitro* (**Fig 4**). To analyze whether the inhibitory effects persist after restimulation with antigen, $CD4^+$ T cells were isolated from mice injected with DC-OVA and DC-OVA-IL-10, respectively, and restimulated with OVA-pulsed epidermal cells. After 3 d the

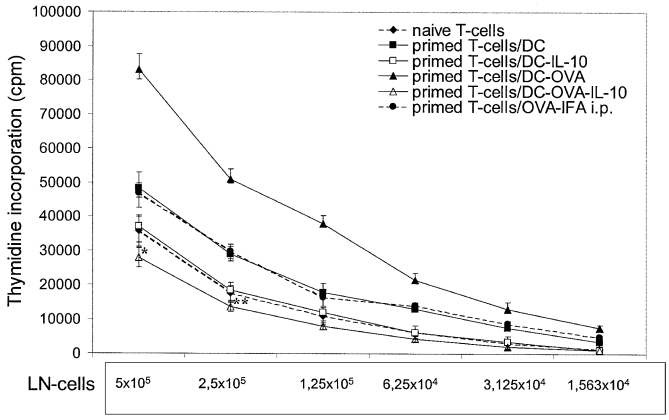


**Figure 4. IL-10 DC downmodulate preexisting OVA-specific immune responses *in vivo*.** $CD4^+$ T cells from DO11.10 mice that had been immunized with OVA peptide in IFA *in vivo* as described were transferred to syngeneic BALB/c mice. Two days later recipients were treated with either IL-10-treated or untreated, OVA-pulsed as described. Lymph node cells from recipients were prepared after 8 d. Proliferation was induced by addition of $OVA^{323–339}$ peptide to the cultures and thymidine incorporation was determined after 3 d. Results shown are representative of five experiments with five mice per group. Groups are background proliferation of lymph nodes from naive transgenic animals stimulated with OVA peptide *in vitro* (naive $CD4^+$ T cells), mice receiving primed $CD4^+$ T cells and unpulsed DC (primed T cells/DC), mice receiving primed $CD4^+$ T cells and DC-OVA (primed T cells/DC-OVA), mice receiving primed $CD4^+$ T cells and DC-IL-10 (primed T cells/DC-IL-10) and mice receiving primed $CD4^+$ T cells and DC-OVA-IL-10 (primed T cells/DC-OVA-IL-10). Error bars represent SEM. *p $<0.05$ and **p $<0.005$ *versus* DC control. Statistical significance was determined by *t* test.

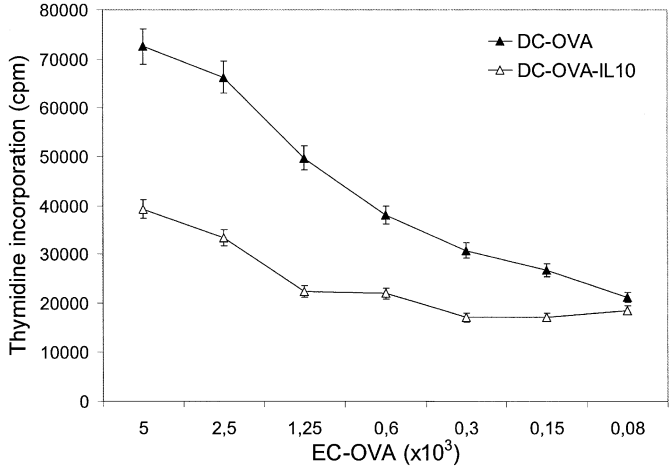


**Figure 5. OVA-pulsed IL-10-treated DC induce antigen-specific tolerance in sensitized T cells *in vivo*.** $CD4^+$ T cells from DO11.10 mice that had been immunized with OVA peptide in IFA *in vivo* as described were transferred to syngeneic BALB/c mice. Two days later recipients were treated with either IL-10-treated or untreated, OVA-pulsed or unpulsed DC as described. $CD4^+$ T cells were isolated from lymph node and spleen cells from recipients after 8 d and were restimulated *in vitro* with OVA-pulsed epidermal cells. After a rest period and addition of IL-2 to the cultures (2 U per ml) $CD4^+$ T cells were restimulated with OVA-pulsed epidermal cells and thymidine incorporation was determined after 3 d. Results shown are representative of five experiments with five mice per group. Groups are mice receiving primed $CD4^+$ T cells and DC-OVA and mice receiving primed $CD4^+$ T cells and DC-OVA-IL10. Error bars represent SEM.

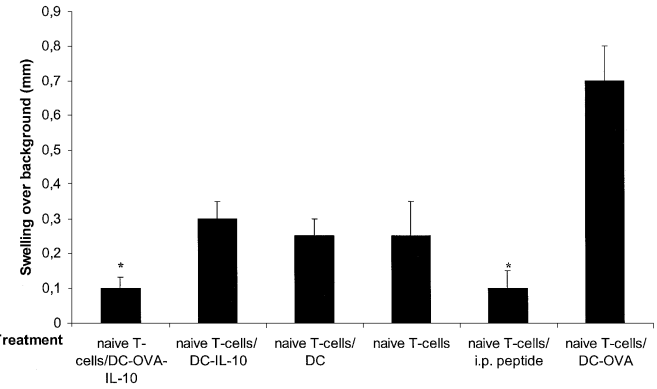


**Figure 6. OVA-pulsed IL-10-treated DC prevent induction of OVA-specific footpad swelling.** $CD4^+$ T cells from DO11.10 mice were transferred to syngeneic BALB/c mice. Two days later recipients were treated with either IL-10-treated or untreated, OVA-pulsed or unpulsed DC as described. Eight days later mice were immunized with $OVA^{323–339}$ in IFA subcutaneously. Ten days later, mice were injected into the footpad with 30 μg $OVA^{323–339}$ in IFA intradermally, or just IFA on the contralateral foot. Footpad swelling was determined 48 h later and was calculated by deducting background swelling of IFA-injected footpads from specific swelling reaction of the contralateral foot. Results shown are representative of three sets of experiments with five mice per group. Groups are mice receiving OVA peptide intraperitoneally (i.p. peptide), mice receiving naive $CD4^+$ T cells and DC-OVA (naive T cells/DC-OVA), mice receiving naive $CD4^+$ T cells and DC-OVA-IL-10 (naive T cells/DC-OVA-IL-10), mice receiving naive $CD4^+$ T cells and DC-IL-10 (naive T cells/DC-IL-10), mice receiving naive $CD4^+$ T cells and unpulsed DC (naive T cells/DC), and mice receiving only naive $CD4^+$ T cells (naive T cells). Error bars indicate SEM. *p $<0.05$ *versus* DC control. Statistical significance was determined by *t* test.

medium was supplemented with 2 U per ml IL-2. After a rest period of 8 d equal numbers of OVA-TCR transgenic $CD4^+$ T cells were restimulated with OVA-pulsed epidermal cells. $CD4^+$ T cells from mice injected with DC-OVA-IL-10 after reconstitution with sensitized $CD4^+$ DO11.10 T cells remained impaired in their capacity to proliferate (**Fig 5**). $CD4^+$ T cells of mice injected with DC or DC-IL-10 were driven into cell death by *ex vivo* restimulation. In summary these data indicate not only that IL-10-treated DC are able to suppress the function of preactivated $CD4^+$ transgenic T cells but that these effects persist after repeated antigen restimulation.

**DTH responses to OVA are suppressed in mice injected with IL-10-treated, OVA-pulsed DC** To look for an *in vivo* correlate of our *in vitro* findings, we performed classical DTH experiments with OVA peptide in IFA. Animals that had been either prophylactically or therapeutically treated as outlined above or mice that had been control-treated were injected intradermally with the immunogenic OVA peptide in IFA. Contralateral limbs just received IFA to determine background swelling responses. Forty-eight hours later footpad swelling reactions were measured. In mice that had been prophylactically treated with DC-OVA-IL-10, footpad swelling to OVA peptide was significantly suppressed compared to the control group of mice that had received DC or DC-IL-10 (**Fig 6**). Omission of antigen in the *in vivo* treatment resulted in swelling reactions similar to those observed in animals reconstituted with naive T cells. Mice that had received DC-OVA showed strong swelling responses. Similar results were obtained when DTH reactions were performed in a therapeutic setting (**Fig 7**). Analysis of DTH reactions in mice injected with DC-OVA-IL-10 showed impaired immune responses after repeated *in vivo* restimulation compared to mice injected with DC-OVA (**Fig 8**). In lymphocyte and splenocyte populations of mice injected with DC or DC-IL-10 no OVA-TCR transgenic T cells could be detected after repeated antigen restimulation *in vivo*. This might account for the small or absent swelling response seen in these groups after antigen inocculation into the footpad (**Fig 8**).

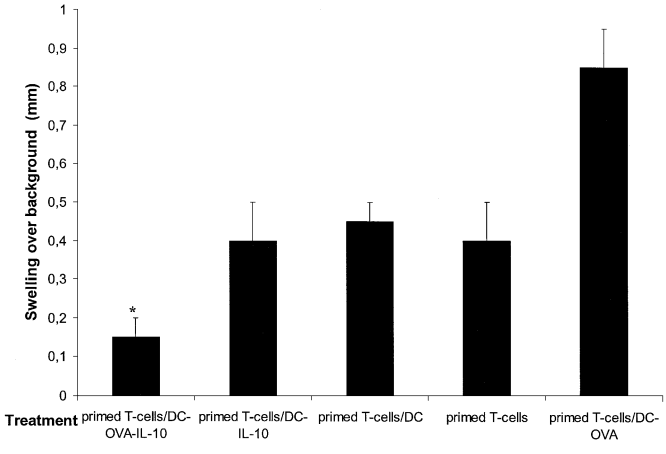


**Figure 7. OVA-pulsed IL-10 DC downmodulate OVA-specific footpad swelling in mice injected with presensitized T cells *in vivo*.** $CD4^+$ T cells from DO11.10 mice that had been immunized with OVA peptide *in vivo* as described were transferred to syngeneic BALB/c mice. Two days later recipients were treated with either IL-10-treated or untreated, OVA-pulsed or unpulsed DC as described. Eight days later, mice were injected into the footpad with 30 μg $OVA^{323–339}$ in IFA intradermally, or just IFA on the contralateral foot. Footpad swelling was determined 48 h later. Results shown are representative of three sets of experiments with five mice per group. Groups are mice receiving primed $CD4^+$ T cells and DC-OVA (primed T cells/DC-OVA), mice receiving primed $CD4^+$ T cells and DC-OVA-IL10 (primed T cells/DC-OVA-IL10), mice receiving primed $CD4^+$ T cells and DC-IL10 (primed T cells/DC-IL10), mice receiving primed $CD4^+$ T cells and unpulsed DC (primed T cells/DC), and mice receiving just primed $CD4^+$ T cells (primed T cells). Error bars indicate SEM. *$p < 0.05$ *versus* DC control. Statistical significance was determined by *t* test.

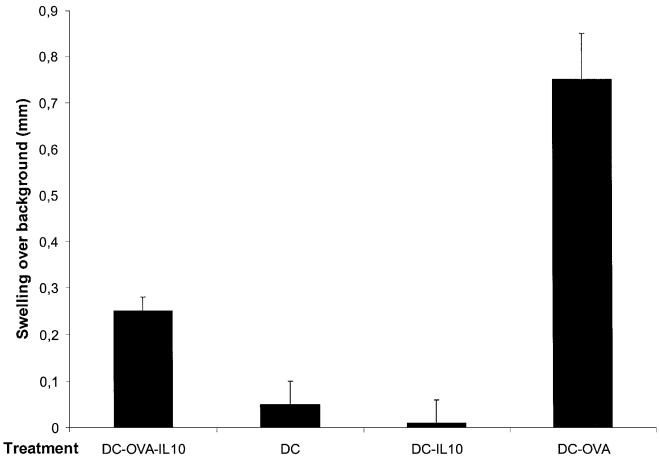


**Figure 8. IL-10-treated DC inhibit footpad swelling after antigen restimulation *in vivo*.** $CD4^+$ T cells from DO11.10 mice that had been immunized with OVA peptide in IFA *in vivo* as described were transferred to syngeneic BALB/c mice. Two days later recipients were treated with either IL-10-treated or untreated, OVA-pulsed or unpulsed DC as described. Mice were injected with $OVA^{323–339}$ in IFA subcutaneously after 8 d. Ten days later mice were injected into the footpad with 30 μg $OVA^{323–339}$ in IFA intradermally, or just IFA on the contralateral foot. Footpad swelling was determined 48 h later. Results shown are representative of five experiments with five mice per group. Groups are mice receiving unpulsed DC, mice receiving DC-IL10, mice receiving DC-OVA, and mice receiving DC-OVA-IL10.

In summary, these data indicate that application of IL-10-treated DC to OVA transgenic mice abrogates DTH reactions to OVA *in vivo*.

## DISCUSSION

IL-10 has been characterized as a modulator of APC function in many different systems. It was shown that IL-10 downmodulates the accessory functions of immature DC and monocytes, but not B cells. The inhibitory effects of IL-10 were due to a down-regulation of MHC class II molecules and costimulatory molecules such as CD80, CD86, or ICAM-1 (de Waal *et al*, 1991; 1992a; Hsu *et al*, 1992; Willems *et al*, 1994). The effects of IL-10 were distinct for different subtypes of APC and also showed species dependency, however (Enk *et al*, 1993; Steinbrink *et al*, 1997). Whereas the expression of MHC class II molecules and costimulatory signals was inhibited on both murine and human monocytes, various subtypes of DC were differentially affected by IL-10. Human-blood-derived DC showed reduced expression of MHC class II and CD80/CD86, whereas no such markers were affected on murine Langerhans cells (Enk *et al*, 1993; Steinbrink *et al*, 1997). In general mature DC were resistant to IL-10 (Steinbrink *et al*, 1997).

Studies with murine Langerhans cells and human-blood-derived DC have shown that pretreatment with IL-10 converts the accessory functions from the induction of proliferation to anergy induction. This was demonstrated for $CD4^+$ and $CD8^+$ T cells (Steinbrink *et al*, 1997). In this study we analyzed the effect of IL-10 treatment on the expression of MHC class II and costimulatory molecules on murine BM-DC and established a system to verify the *in vitro* results *in vivo*. We demonstrated in an *ex vivo* transfer system using $CD4^+$ T cells from OVA-TCR transgenic mice in syngeneic BALB/c recipients that IL-10-treated DC are able to prevent the induction of sensitization in transgenic $CD4^+$ T cells. Following sensitization with immunogenic peptide in IFA, lymph node cells from recipients treated with IL-10-treated, OVA-pulsed DC showed a proliferation similar to that of a tolerance control group that just received the immunogenic peptide in IFA intraperitoneally. In contrast, lymph node cells from recipients treated with normal OVA-pulsed DC mounted a strong proliferative response upon stimulation of the cells with immunogenic peptide *in vitro*. More importantly, IL-10-treated DC were also able to suppress ongoing immune responses in presensitized T cells. For this, $CD4^+$ T cells from *in vivo* sensitized transgenic animals were tranferred into syngeneic recipients. When recipients were injected with IL-10-treated OVA-pulsed DC, lymph node T cells that were prepared and restimulated with immunogenic peptide *in vitro* showed a significantly lower T cell proliferation compared with the control group. Even after repeated antigen restimulation *in vivo* and *in vitro* full proliferative capacity of DC-OVA-IL10-treated cells cannot be restored.

Our results are in agreement with earlier work by Groux *et al* (1996). Besides describing a direct tolerizing effect of IL-10 on $CD4^+$ T cells in their earlier work, these authors have recently developed a transgenic mouse system where the effect is achieved by the expression of human IL-10 under the control of the murine MHC class II Ea promoter (Groux *et al*, 1999). In these animals, IL-10 is secreted by APC such as macrophages or DC. Although no gross abnormalities in serum Ig levels or peripheral lymphocyte populations were detectable in these mice, transgenic animals failed to mount detectable T or B cell immune responses to OVA. In addition, these animals were highly susceptible to infection with intracellular pathogens like Listeria monocytogenes or Leishmania major. Although the exact reason for the poor immune response in IL-10 transgenic mice remained undetermined, T cell anergy and the induction of regulatory (Tr1-type) T cells were discussed as possible mechanisms.

In human disease, there is also ample evidence to support our findings that IL-10 inhibits DC function *in vivo*. Mitra *et al* (1995) have shown that the secretion of IL-10 in human psoriatic plaques inhibits the APC function of human dermal DC. The effect was mediated by an inhibition of the expression of costimulatory molecules such as CD86, CD80, as well as HLA-DR molecules. For patients with malignant melanoma it has been demonstrated

that high amounts of IL-10 in patients' sera is associated with a poor prognosis (Dummer *et al*, 1995). Also, secretion of IL-10 by melanoma cells was shown to convert local DC function from induction of tumor immunity to the induction of T cell anergy (Dummer *et al*, 1996). Similar results were obtained for lung cancer, where T-cell-derived IL-10 suppressed both APC and T cell functions (Smith *et al*, 1994).

On the other hand, the inhibitory effect of IL-10-treated DC *in vivo* makes these cells an attractive tool for future studies in experimental murine and hopefully human autoimmune diseases. The exact nature of the immune-modulatory effect exerted by IL-10-treated DC still needs to be analyzed (e.g., suppression versus anergy induction). Nevertheless there is a clear difference between OVA-pulsed IL-10-treated DC and control DC. In mice injected with control DC (DC or DC-IL10) no OVA-TCR transgenic T cells can be detected after repeated restimulation with OVA peptide *in vivo*, whereas $CD4^+$ transgenic T cells can be isolated following antigen restimulation in mice injected with OVA-pulsed IL-10-treated DC. In addition, $CD4^+$ T cells of mice injected with control DC were driven into cell death by secondary stimulation *in vitro*. Thus OVA-pulsed IL-10-treated DC seem to induce T cell survival without T cell activation. The inhibitory effect of DC-IL10 most likely is not a direct effect of IL-10 on $CD4^+$ T cells, as no IL-10 was released by IL-10-treated DC (not shown). Studies to better define the nature of the tolerizing T cell response induced by IL-10-treated APC and the molecules involved in this reaction are currently in progress in our laboratory.

*Supported by Deutsche Forschungsgemeinschaft SFB 548-A3.*

## REFERENCES


Banchereau J, Steinman RM: Dendritic cells and the control of immunity. *Nature* 392:245-248, 1998

Dummer W, Becker JC, Schwaaf A, Leverkus M, Moll T, Brocker EB: Elevated serum levels of interleukin-10 in patients with metastatic malignant melanoma. *Melanoma Res* 5:67-78, 1995

Dummer W, Bastian BC, Ernst N, Schanzle C, Schwaaf A, Brocker EB: Interleukin-10 production in malignant melanoma: preferential detection of IL-10-secreting tumor cells in metastatic lesions. *Int J Cancer* 66:607-614, 1996

Enk AH, Angeloni VL, Udey MC, Katz SI: Inhibition of Langerhans cell antigen-presenting function by IL-10. A role for IL-10 in induction of tolerance. *J Immunol* 151:2390-2396, 1993

Enk AH, Jonuleit H, Saloga J, Knop J: Dendritic cells as mediators of tumor-induced tolerance in metastatic melanoma. *Int J Cancer* 73:309-314, 1997

Gastl GA, Abrams JS, Nanus DM, *et al*: Interleukin-10 production by human carcinoma cell lines and its relationship to interleukin-6 expression. *Int J Cancer* 55:96-103, 1993

Groux H, Bigler M, de Vries JE, Roncarolo MG: Interleukin-10 induces a long-term antigen-specific anergic state in human CD4+ T cells [see comments]. *J Exp Med* 184:19-28, 1996

Groux H, Cottrez F, Rouleau M, *et al*: A transgenic model to analyze the immunoregulatory role of IL-10 secreted by antigen-presenting cells. *J Immunol* 162:1723-1734, 1999

Hsu DH, Moore KW, Spits H: Differential effects of IL-4 and IL-10 on IL-2-induced IFN-gamma synthesis and lymphokine-activated killer activity. *Int Immunol* 4:563-571, 1992

Kearney ER, Pape KA, Loh DY, Jenkins MK: Visualization of peptide-specific T cell immunity and peripheral tolerance induction *in vivo*. *Immunity* 1:327-333, 1994

Kim J, Modlin RL, Moy RL, Dubinett SM, McHugh T, Nickoloff BJ, Uyemura K: IL-10 production in cutaneous basal and squamous cell carcinomas. A mechanism for evading the local T cell immune response. *J Immunol* 155:2240-2248, 1995

Merlo A, Juretic A, Zuber M, *et al*: Cytokine gene expression in primary brain tumours, metastases and meningiomas suggests specific transcription patterns. *Eur J Cancer* 29A:2118-2121, 1993

Mitra RS, Judge TA, Nestle FO, Turka LA, Nickoloff BJ: Psoriatic skin-derived dendritic cell function is inhibited by exogenous IL-10. Differential modulation of B7-1 (CD80) and B7-2 (CD86) expression. *J Immunol* 154:2668-2674, 1995

Murphy KM, Heimberger AB, Loh DY: Induction by antigen of intrathymic apoptosis of CD4+CD8+TCRlo thymocytes *in vivo*. *Science* 250:1720-1723, 1990

Pisa P, Halapi E, Pisa EK, *et al*: Selective expression of interleukin 10, interferon gamma, and granulocyte–macrophage colony-stimulating factor in ovarian cancer biopsies. *Proc Natl Acad Sci USA* 89:7708-7713, 1992

Schuler G, Steinman RM: Murine epidermal Langerhans cells mature into potent immunostimulatory dendritic cells *in vitro*. *J Exp Med* 161:526-536, 1985

Smith DR, Kunkel SL, Burdick MD, Wilke CA, Orringer MB, Whyte RI, Strieter RM: Production of interleukin-10 by human bronchogenic carcinoma. *Am J Pathol* 145:18-29, 1994

Steinbrink K, Wolfl M, Jonuleit H, Knop J, Enk AH: Induction of tolerance by IL-10-treated dendritic cells. *J Immunol* 159:4772-6684, 1997

Steinbrink K, Jonuleit H, Muller G, Schuler G, Knop J, Enk AH: Interleukin-10-treated human dendritic cells induce a melanoma-antigen- specific anergy in CD8(+) T cells resulting in a failure to lyse tumor cells. *Blood* 93:1634-1646, 1999

Steinman RM, Young JW: Signals arising from antigen-presenting cells. *Curr Opin Immunol* 3:361-371, 1991

de Waal M, Haanen J, Spits H, *et al*: Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *J Exp Med* 174:915-923, 1991

de Waal M, Yssel H, Roncarolo MG, Spits H, de Vries JE: Interleukin-10. *Curr Opin Immunol* 4:314-331, 1992a

Willems F, Marchant A, Delville JP, *et al*: Interleukin-10 inhibits B7 and intercellular adhesion molecule-1 expression on human monocytes. *Eur J Immunol* 24:1007-1014, 1994

Young JW, Koulova L, Soergel SA, Clark EA, Steinman RM, Dupont B: The B7/BB1 antigen provides one of several costimulatory signals for the activation of CD4+ T lymphocytes by human blood dendritic cells *in vitro* [published erratum appears in *J Clin Invest* 91(4):1853, 1993]. *J Clin Invest* 90:229-235, 1992